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THE EFFECT OF A PROBIOTIC PREPARATION ON THE WEIGHT GAIN, IMMUNE SYSTEM AND HEALTH OF CALVES

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Abstract

The objective of the study was to ascertain the impact of a bacteria-based probiotic preparation on both the weight gain and overall health of calves. This investigation was carried out on Polish Holstein-Friesian calves ranging in age from 2 to 60 days. The calves were divided into two groups: group 1 (the control group) and group 2 (the experimental group which received the probiotic preparation). The test included the administration of 20 ml of the probiotic preparation to calves twice daily. During the experiment, samples of blood and excrement were collected for laboratory testing. Specimens were collected on days 7, 30, and 60 after birth with a margin of error of +/-2 days. The obtained blood underwent morphological analysis, which included determining the quantities of immunoglobulins in the IgA, IgM, and IgG classes. Additionally, microbiological and parasitological tests were conducted on the faeces. A comprehensive assessment was conducted of the animals' health condition and the amount of weight they gained throughout the trial. According to the findings, the calves that were given the probiotic preparation (group 2) exhibited an increase of around 12% in their daily weight gain and had a higher ultimate body mass. During the research period, irregular variations were noted in the quantity of leukocytes and haemoglobin in the subjects. Both groups exhibited a rising trend in erythrocyte count and haematocrit. The group that received the probiotic product had modestly elevated serum concentrations of IgG, IgM, and IgA. The highest levels of IgG were seen in the plasma in both groups on day 7. The results of the faecal microbiological tests revealed that both groups 1 and 2 had the greatest total bacterial counts on day 7 of life, which significantly declined at later time points ($p \leq 0.05$). Comparable correlations were identified for E. coli and Clostridium perfringens. The sample under analysis included eggs of gastrointestinal nematodes belonging to the order Strongylida, with a mean invasion intensity of 20 to 40 eggs per gramme (EPG). Additionally, coccidia of the genus Eimeria sp. were also present, in quantities of 10-30 oocysts per gramme (OPG). Cryptosporidium sp. oocysts were not found in any of the samples examined during the parasitological analysis of the faeces. The findings suggest that the administration of the probiotic preparation positively influenced the weight increase and final body mass of the calves. Additionally, it had a positive impact on the physical characteristics of blood, levels of immunoglobulins, and the composition of the gastrointestinal microbiota and parasitology.

Keywords: calves, probiotic preparation, weight gain, morphological parameters, immune antibodies

Introduction

The most challenging phase in the process of raising calves is the time until the end of the third month of their lives (Stenzel et al., 2000). During the first months of a calf's life, there is a significant increase in tissue and organ growth, along with the development of the foregut and the build-up of immunity. Research shows the standard of farm management significantly influences the incidence of calf illness and death. Although there have been notable improvements in the rearing and servicing of dairy calves in the United States, the pre-weaning mortality rate remains around 10% (Hulbert and Moisá, 2016). Conversely, the rate of calf mortality in the Netherlands during the first year of life is roughly 16.5%. The death rate in postnatal calves (≤14 days) is 3.3%, in early weaners (15 to 55 days) it is 4.5%, and in weaners older than 56 days – 3.1% (Santman-Berends et al., 2019). This may be attributed to substantial environmental fluctuations occurring during this period, along with the diminished immunity seen in new-born calves. The intestinal microflora plays a significant role in the appropriate growth and development of animals. The intestinal microflora governs the processes of digestion, metabolism, and the functioning of the immune system. Disruption of the body's equilibrium diminishes natural immunity, making individuals more susceptible to diseases, particularly those caused by viruses (Guarner and Malagelada, 2003). Research shows that probiotics and prebiotics possess considerable potential for enhancing animal production (Uyeno et al., 2015). Probiotics serve various purposes, such as protecting against enteropathic disorders, enhancing feed utilisation and weight gain, impacting the composition of the gastrointestinal microflora, maintaining intestinal health, and promoting animal well-being through metabolite stability and immune system modulation (Timmerman et al., 2005; Lesmeister et al., 2004). They could potentially serve as a substitute for antibiotics (Allen et al., 2013), which are problematic due to safety concerns regarding antibiotic resistance, the release of antibiotics into the environment, and the persistence of antibiotic residues in animal products (Martínez-Vaz et al., 2014; Mc Allister et al., 2018). The precise processes by which probiotics exert their effects have not been definitively established. However, it is hypothesised that these bacteria decrease the pH in the large intestine by producing lactic acid, which in turn inhibits the development of harmful bacteria (Riddell et al., 2010). Probiotic pills are highly recommended throughout the early stages of a calf's life. The digestive system of new-born calves is believed to be free of microorganisms, and colonisation of the gastrointestinal tract starts soon after delivery. However, there have been studies suggesting the potential colonisation of the calf's gut as early as the foetal stage. Research on the perinatal microbiome of new-born calves reveals the existence of a limited quantity of microorganisms. The microbial flora consists of Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes. Within the first day after birth, the gastrointestinal system becomes inhabited by environmental bacteria such as Escherichia, Shigella, and Clostridia. The microbiome's diversity starts to develop around one week after birth (Alipour et al., 2018; Villot et al., 2020). During adolescence, the large intestine develops a sophisticated and constantly evolving microbial community, characterised by a high concentration of viable bacteria. An analysis of calf intestinal bacterial communities using molecular techniques has shown that these communities experience dynamic fluctuations throughout the first 12 weeks of life (Uyeno et al., 2010).

The impact and effectiveness of probiotics rely on the specific strains that are used (Bhat and Kapila, 2017). *Lactobacillus casei* 1k 2 ATCC 7469 is a strain that synthesises a protein responsible for transporting folate (Henderson et al., 1985). Other bacteria from the *Lactobacillus* and *Bifidobacterium* genera have the ability to produce folic acid (Rossi et al., 2011) and regulate pyruvate levels (Benito de Cárdenas et al., 1987). The levels and conversion of pyruvate to lactates can be negatively affected by stress during birth and weaning. This

syndrome in calves may lead to issues with detoxification and low ATP levels in the mitochondria, resulting in body weakness and impaired immune function. The *Lactobacillus casei* 1k 2 ATCC 7469 strain is highly recommended for calves because it plays a crucial role in the transportation of folic acid. Folic acid is engaged in several metabolic processes inside the organism, such as the metabolism of certain amino acids, the production of purines and pyrimidines, and the synthesis of methionine. Sufficient quantities maintain the hematopoietic system and the epithelium of the digestive tract in a healthy state. The health of the young organism is impacted by deficiencies that arise from situations of increased intake, such as diarrhoea and anaemia. Research conducted on young animals demonstrates that calves or lambs might have a severe folic acid shortage throughout the post-weaning phase until their rumen function is fully developed (Dumoulin et al., 1991). *Lactobacillus plantarum* ATCC 8014 is involved in the conversion and metabolism of D-biotin, as well as the transport and accumulation of biotin. Biotin is crucial for the metabolism of fatty acids and glucose, and it also affects the creation of keratin fibres and the differentiation of epidermal, horn, and hoof tissue. The precise molecular processes underlying the relationship between the mammalian host and its microbiota remain poorly understood. Recent research indicates that metabolites generated by gut bacteria have the ability to impact the expression of genes in mammals by means of epigenetic modification (Ye et al., 2017). Although there are many probiotic microbial products available for purchase, it is important to customise probiotic treatment to suit the specific requirements of each farm and animal (Raabis et al., 2019). Research indicates that the gut microbiome has a significant impact on immune system activation, and an uneven distribution of bacteria in the gastrointestinal tract might potentially contribute to the onset of many illnesses (Bouskra et al., 2008). Further research is required to explore the possible use of microbes in the process of raising calves. Therefore, a study was conducted to assess the impact of a probiotic formulation on the well-being and weight gain of calves during the first 60 days of their life.

Materials and methods

The study was carried out on a dairy cow farm forming part of a Research Facility in Kobylany, focusing on 20 calves of the Polish Holstein-Friesian breed, ranging in age from 2 to 60 days. The calves were chosen for the trial based on the colostrum's IgG levels, which were determined using a colostrometer. The experiment chose calves delivered by cows with milk IgG level fluctuating between 50-80 $g \cdot l^{-1}$. This ensured a standardised experimental material with consistent resistance and adaptability. The calves were categorised into two groups: group 1, or the control group, and group 2, or the experimental group. The animals in the experimental group were given a probiotic bacteria preparation, with a dosage of 20 ml injected twice daily. The preparation, as instructed by the manufacturer, included the following components: *Saccharomyces cerevisiae* at a concentration of 3.0x104 cfu/ml IFO 0203, mesophilic lactic fermentation bacteria (*Lactobacillus casei* 1k 2 ATCC 7469, *Lactobacillus plantarum* 1 k 2 ATCC 8014) at a concentration of 5.0x107 cfu/ml, and cane molasses. Feeding began on the second day and continued until the sixtieth day. In line with the farm's management policies, calves were moved to the calving house and provided with milk substitute at the feeding station from day 3 after birth until they reached 60 days of age. The farm used dosage management to control the intake of milk substitute by the calves. Calves aged up to 30 days used 6 litres of preparation per day (135 grammes of preparation per litre of water, which is equivalent to 810 grammes a day). Older calves consumed 8 litres of preparation per day (1080 grammes per day). From the beginning, the animals were provided with uninterrupted water supply and were taught to consume feed – first from the station and then concentrated feed. The calves' diet, which consisted of milk substitute preparation, muesli, and CJ mix, had

a protein concentration ranging from 20% to 22%. The milk substitute consisted of 20% crude protein, 10% crude ash, 18% crude oils and fats, 1.9% lysine, 0.9% calcium, 0.7% phosphorus, and 0.8% sodium. The supplementary feed provided to the calves consisted of maize, dried alfalfa, barley, heat-treated post-extraction huskless soybean meal, beetroot molasses, calcium carbonate, sodium chloride, monocalcium phosphate, and magnesium phosphate. The mixture's chemical makeup consisted of 18.2% crude protein, 8.40% crude ash, 2.50% crude fat, and 7.30% crude fibre. The weight increase of the animals was assessed by measuring their weight at birth and again at 60 days of age. Throughout the trial, a daily evaluation of the well-being of the calves was conducted. The experiment's timing was adjusted to align with the activities taking place on the farm. The material collected on the farm, including blood and excrement, underwent frequent laboratory analysis to keep track of the animals' health condition via veterinarian screening. The material was collected in accordance with established policies. Blood was sampled from the zygomatic vein and dispatch promptly for morphological testing using a blood smear. A portion of the blood was separated by centrifugation, and the levels of serum immunoglobulins (namely, IgG, IgA, and IgM classes) were measured by the immunoenzymatic ELISA method. Faecal samples were obtained from the rectum for testing purposes. Following collection, the refrigerated samples were promptly sent to the laboratory for microbiological analysis. Each faecal sample was weighed to 10 grammes and then combined with 90 cubic centimetres of physiological fluid, resulting in a starting dilution of 0.1 cubic centimetres. Microbiological mediums were used to ascertain the presence and quantity of certain bacteria groups. The TSA medium was used to quantify the total number of aerobic mesophilic bacteria in a 24-hour culture at a temperature of 37 degrees Celsius. The SS medium is a type of agar media that was used to selectively cultivate *Salmonella sp*. and *Shigella* bacteria. The culture was incubated for 24 hours at a temperature of 37 degrees Celsius. Chapman's medium was inoculated with a 24-hour culture of *Staphylococcus sp*. and incubated at a temperature of 37 degrees Celsius. Agar endo was inoculated with a 24-hour culture of *Escherichia coli* and incubated at a temperature of 44 degrees Celsius. The Wilson-Blair medium was used to cultivate *Clostridium perfringens* for 24 hours at a temperature of 37 degrees Celsius. The faeces were subjected to parasitological investigation using the McMaster technique with centrifugation to identify gastrointestinal parasites (Roepstorff and Nansen, 1998). Additionally, a modified Ziehl-Neelsen method was used to determine the presence of *Cryptosporidium sp.* (Henriksen and Pohlenz, 1981). The Agricultural University of Kraków conducted microbiological tests in the Department of Microbiology and parasitological exams in the Department of Environmental Zoology, following established testing protocols. On days 7, 30, and 60 of calf rearing, haematological, microbiological, and parasitological tests were performed on the calf excrement.
The obtained results were analysed by ANOVA and Duncan's test using Statistica 12 (StatSoft, Poland 2013).

Results

The experiment revealed that over a period of 60 days, the group that received the probiotic preparation had greater weight increases in the calves. In group 1, the average daily gain was 480 g, while in group 2 it was almost 12% higher than the control group, averaging 536 g-d^{-1} (Table 1). The calf blood morphological indicators were analysed and the findings are shown in Table 2. During the study period, there were inconsistent variations in the levels of leukocytes and haemoglobin. Both groups exhibited a rising trend in the quantity of erythrocytes and haematocrits. The white blood cell counts in both groups remained within the top ranges of established standards (Baumgartner, 2005). In the control group, there was a statistically significant ($p \leq 0.05$) rise in white blood cell (WBC) counts above the recognised limits on day

30. However, in the following assessment, the WBC levels were within the reference norms. The blood of group 1 (control) calves on day 60 had the largest proportion of lymphocytes, while group II calves had the lowest proportion throughout the first week of life.

Table 1. Effect of probiotic preparation on body weight gain of calves and incidence of diarrhea

Wyszczególnienie Item	Grupa I Group I	Grupa II Group II
Waga urodzeniowa (kg) Birth weight (kg)	41.9a	42.2a
Waga w 60 dniu życia (kg) Weight at 60 days of age (kg)	70.6a	74.4b
Średnie dzienne przyrosty masy ciała (g/dzień) Average daily weight gain (g·day^{-1})	480a	536b
% cieląt z biegunką % of calves with diarrhea	18b	12a

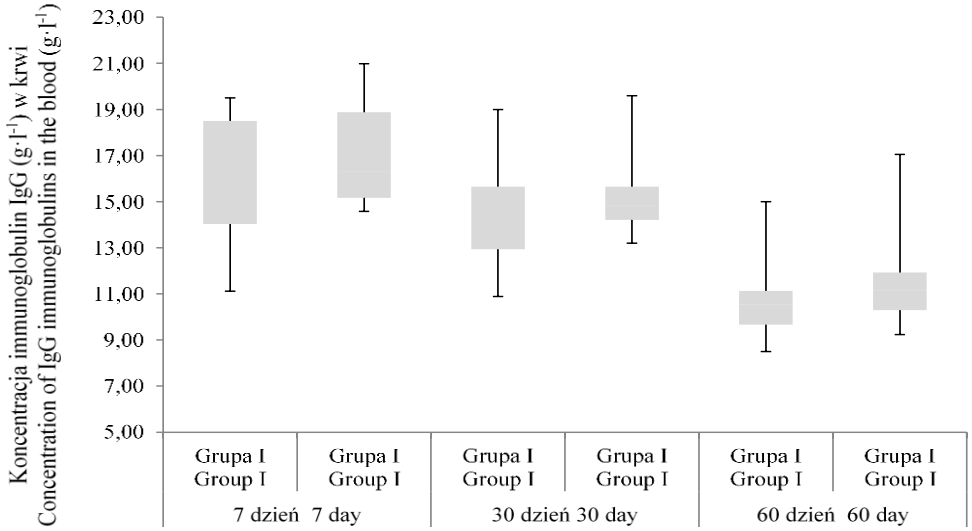
a, b – różnice istotne (p≤0,05)
a, b – significant differences (p≤0.05)

Table 2. Hematological blood parameters in calves

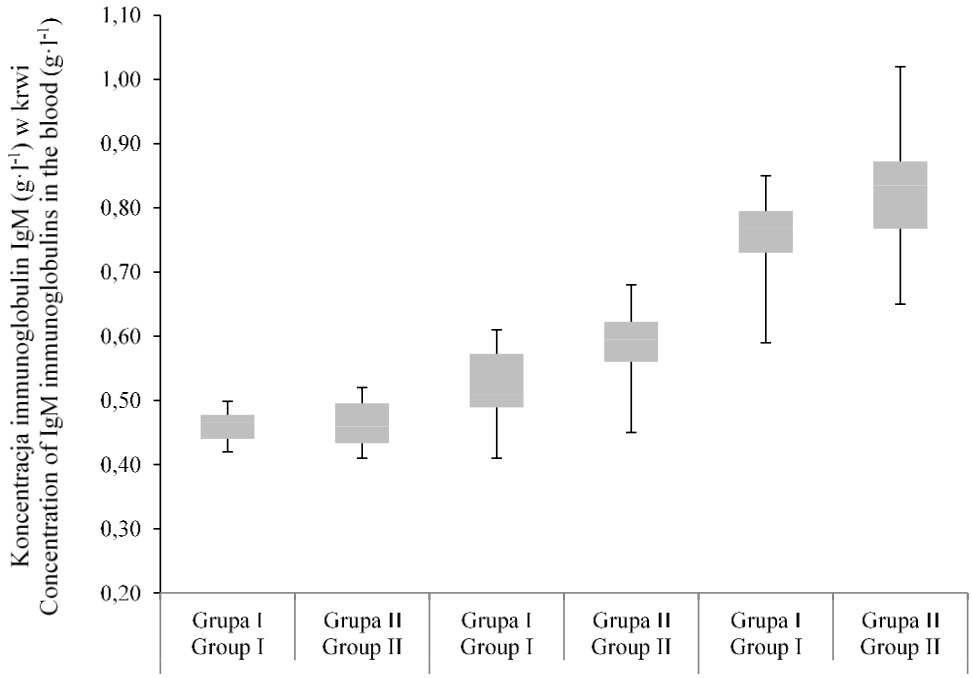
Parametry krwi Blood parameters	Grupa I Group I			Grupa II Group II		
	7*	30	60	7	30	60
WBC – Liczba białych krwinek White blood cells (G·l)	10.65ab	13.18c	9.57a	11.30b	10.43ab	11.40b
RBC – Liczba czerwonych krwinek Red blood cells (T·l^{-1})	7.04a	7.39ab	8.22c	7.59b	7.90b	8.52c
HGB – Poziom hemoglobiny Hemoglobin concentration (mmol·l^{-1})	5.98b	5.55a	6.15b	6.77c	6.00b	6.40bc
Hct – Hematokryt Hematocrit value (l·l^{-1})	0.28a	0.27a	0.29b	0.32c	0.29b	0.30b
MCV – Średnia objętość erytrocytu Mean corpuscular volume (fl)	39.80b	36.55a	35.35a	42.10b	36.70a	35.20a
RDW – Rozpiętość rozkładu wielkości erytrocytów Red blood cell distribution width (%)	16.75c	16.25bc	15.33a	15.43a	15.83b	15.08a
PLT – Płytki krwi Blood platelets (G·l^{-1})	563c	525b	507b	587c	472a	509b
MPV – Średnia objętość płytek krwi Mean platelet volume (fl)	5.00	5.00	5.20	5.00	5.00	5.00
Rozmaz manualny wg Schillinga: Manual blood smear according to Schilling:						
Granulocyty kwasochłonne (%) Eosinophilic granulocytes (%)	1	1	2	1	2	2
Segmentowane neutrofile (%) Neutrophilic granulocytes (%)	40b	44b	16a	56c	34ab	32ab
Limfocyty (%) Lymphocytes (%)	56ab	52a	77c	40a	61b	63b
Monocyty (%) Monocytes (%)	2	2	1	2	2	2
Zasadochłonne bazofile (%) Basophilic granulocytes (%)	1	1	2	1	1	1

a, b, c – significant differences (p≤0.05)
*7, 30 and 60 days after birth

a)


Koncentracja immunoglobulin IgG (g·l-1) w krwi
Concentration of IgG immunoglobulins in the blood (g·l-1)
23,00
21,00
19,00
17,00
15,00
13,00
11,00
9,00
7,00
5,00
Grupa I Group I
Grupa I Group I
Grupa I Group I
Grupa I Group I
Grupa I Group I
Grupa I Group I
7 dzień 7 day
30 dzień 30 day
60 dzień 60 day

b)


Koncentracja immunoglobulin IgM (g·l-1) w krwi
Concentration of IgM immunoglobulins in the blood (g·l-1)
1,10
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
Grupa I Group I
Grupa II Group II
Grupa I Group I
Grupa II Group II
Grupa I Group I
Grupa II Group II

c)

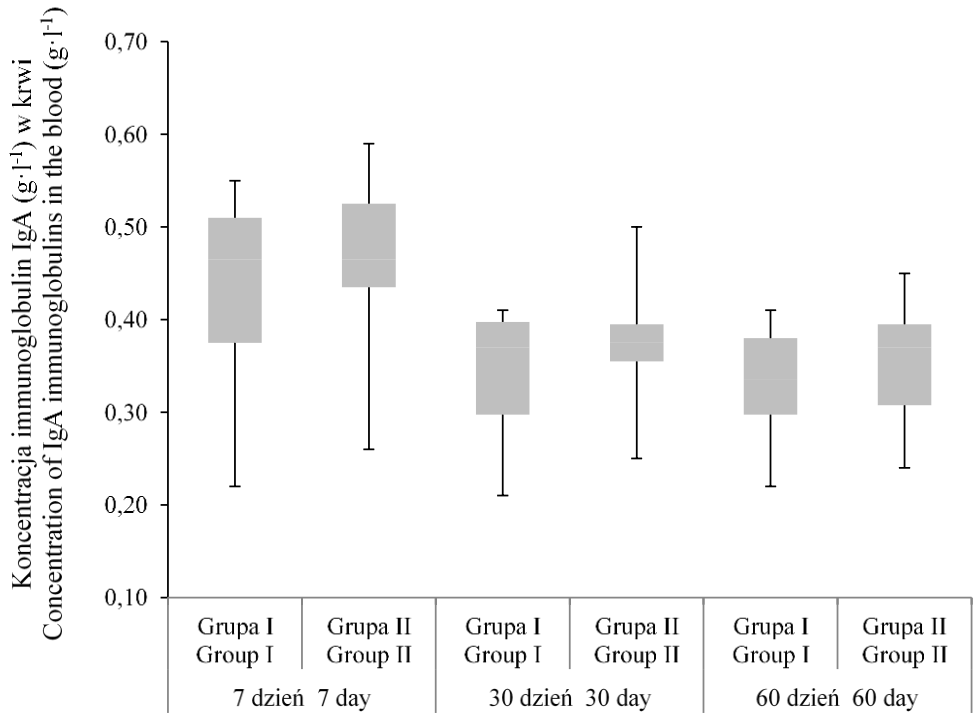


Figure 1. The immunoglobulin concentration in blood of calves ($g \cdot l^{-1}$)

Different erythrocyte levels were found depending on the age of the calves. At the beginning of the experiment, both groups exhibited the lowest values, while the highest values were recorded at 60 days of age. The group that received the probiotic preparation had the greatest concentration of haemoglobin (6.77 $mmol\text{-}l^{-1}$) and haematocrit value (0.32 $l\text{-}l^{1}$) on day 7 of life. On the other hand, the control group had the lowest values on day 30, with haemoglobin concentration of 5.55 $mmol\text{-}l^{-1}$ and haematocrit value of 0.27 $l\text{-}l^{1}$. The red blood cell parameters MCV, MCH, and MCHC exhibited a decline in correlation with the age of the calves. The peak values were seen on day 7, however there were no statistically significant variations between days 30 and 60.

Elevated serum levels of IgG, IgM, IgA were found in the group receiving the probiotic preparation (Figure 1a, b, c). Both groups exhibited peak levels of IgG on day 7, followed by a gradual decline in subsequent measurements. The study revealed similar correlations for IgA levels, with the maximum concentration seen on day 7, while remaining rather steady on days 30 and 60. An elevation in IgM concentration was seen in the following days of life.

The faecal microbiological tests revealed that the maximum count of total mesophilic aerobic bacteria was observed when the calves were seven days old in both control group 1 and experimental group 2 (Table 3). A statistically significant decrease ($p \leq 0.05$) in the overall bacterial count was seen in calves on day 30 and day 60. Comparable correlations were identified for *E. coli* and *Clostridium perfringens*. During the first month of life, there was a notable rise in the quantity of *Staphylococcus sp.* bacteria detected in the calves' excrement. *Salmonella sp.* was not detected in any of the faecal samples from calves up to 7 days old (Table 4). Nevertheless, after 60 days of age, both the control and probiotic-treated groups displayed the presence of these bacteria in the collected faeces. However, the control group had a

considerably higher bacterial count compared to the experimental group. The sample under analysis contained eggs of gastrointestinal nematodes belonging to the order *Strongylida*, with an average invasion intensity of 20 to 40 eggs per gramme (EPG). Additionally, eggs of *coccidia* from the genus *Eimeria sp*. were also detected, with an average of 10-30 oocysts per gramme (OPG). No oocysts of *Cryptosporidium sp.* were found in any of the analysed faecal samples during the parasitological testing.

Table 3. Fecal microbiological tests (cfu·1 cm^{-3})

Grupa Group	Czas pobrania Collection time	Ogólna liczba bakterii tlenowych mezofilnych Total no. of aerobic mesophilic bacteria	*Salmonella* sp.	*Shigella*	*E. coli*	*Staphylococcus* sp.	*Clostridium perfringens*
Grupa I Group I	7	284344000d	nw.	624346d	239350e	81767c	134600b
	30	12556667a	17a	487608c	271020d	242661e	1525a
	60	8221667a	106b	183575a	28392b	81433c	1778a
Grupa II Group II	7	112900000c	nw.	585570d	219044e	43256b	212145c
	30	70250000b	0	337800b	188000c	115800d	1500a
	60	31135714b	14a	171944a	10193a	17486a	1427a

a, b, c, d, e – significant diferences (p≤0.05) ; nw. – not detected

Table 4. Fecal parasitological tests

Grupa Group	Czas pobrania Collection time	*Eimeria* sp. (OPG)	Strongylida (EPG)	*Cryptosporidium* sp. (+ / -)
Grupa I Group I	7	0	0	-
	30	0	0	-
	60	30c	40b	-
Grupa II Group II	7	30c	0	-
	30	20b	40b	-
	60	10a	20a	-

a, b, c – significant differences (p≤0.05)

Discussion

In calf raising, certain bacterial cultures are being used more often as probiotics and prebiotics (Schamberger and Diez-Gonzalez, 2002; Tkalcic et al., 2007). Research has demonstrated that microorganisms found in probiotics rapidly reproduce in the gastrointestinal tract. They outcompete harmful strains of *E. coli* and other bacteria that produce toxins. Additionally, probiotics help maintain a stable acidity level in the gastrointestinal tract and decrease the incidence of diarrhoea (Von Buenau et al., 2005; Timmerman et al., 2005). Nevertheless, the findings from studies on the use of probiotics in calves are ambiguous. Research indicates that the administration of probiotics and prebiotics may enhance the ability of animals to digest dry matter, increase their intake of crude protein and amino acids, and improve the absorption of minerals in the intestines (Li et al., 2008; Kong et al., 2011). Probiotics have a proven ability to enhance rearing performance, namely by improving weight growth, feed conversion, and average daily weight gain during the first two weeks of life. Animals have shown a decrease in

the incidence and length of diarrhoea, as reported by Abe et al. (1995) and Timmerman et al. (2005). The prevalence of falls, as well as the occurrence of respiratory illness and diarrhoea, is reduced, leading to enhanced health and increased weight gains in calves (Agarwal et al., 2002; Timmerman et al., 2005). Several researchers have found no beneficial effects associated with the use of probiotics, as shown by Higgenbotham and Bath (1993) and Riddell et al. (2010). The differences might be attributed to factors such as the specific probiotic strains used, the amount of probiotics administered, the nature and content of the feed utilised, or the particular animal species subjected to testing. The positive impact of probiotics on daily weight gain shown in this trial aligns with the findings of previous studies (Lesmeister et al, 2004; Timmerman et al, 2005; Roodposhti and Dabiri, 2012). Lesmeister et al. (2004) observed a rise in the average weight increase over a 24-hour period when calf meals were supplemented with 2% probiotics. Similarly, Roodposhti and Dabiri (2012) discovered a statistically significant increase ($p<0.05$) in the average daily weight gains in calves that were given probiotic, prebiotic, and synbiotic supplements. Timmerman et al. (2005) conducted a study where they fed probiotics to calves for 8 weeks. The results showed a substantial impact on both the average daily weight growth and feeding efficiency of the calves. Probiotics decreased the occurrence and average duration of diarrhoea. Additionally, the usage of milk substitutes with probiotics showed a tendency towards lower death rates. According to the research, probiotics have a more pronounced impact under stressful conditions such as transportation, unfamiliar surroundings, changes in nutrition, or exposure to infectious agents (Timmerman et al., 2005). This is further supported by Riddell et al. (2010), who have proved that probiotic supplementation had no impact on dry matter consumption or average daily weight gain. The authors attribute the absence of disparities in growth and health condition to the possibility that calves housed indoors in a regulated temperature setting with little extra stressors may not exhibit a favourable reaction to probiotics.

The preparation used in our study consisted of a strain of *Saccharomyces cerevisiae*, which has distinctive probiotic properties. Its resilience to acidic conditions, bile, and temperatures up to 600°C allows it to reach the gut and adapt to its surroundings without colonising it (Thorsteinsson et al., 2020; Pais et al., 2020). It aids in the reduction of inflammation by suppressing the synthesis of pro-inflammatory cytokines. It inhibits the proliferation and adhesion of harmful bacteria like *Escherichia coli* and *Salmonella* to the mucous membrane, and it fortifies the intestinal barrier during *Shigella flexneri* infection (Pothoulakis, 2009). *Saccharomyces cerevisiae* produces a particular protease that breaks down exotoxins A and B produced by *Clostridium difficile*, which induce inflammation in the intestines. This protease also digests the receptor binding sites of the toxins (Castagliuolo et al., 1996). Research has identified diverse outcomes resulting from the use of this particular strain. In their study, Villot et al. (2019) discovered that while there was no increase in daily weight gain or feed intake in calves supplemented with *Saccharomyces*, the incidence of diarrhoea was decreased compared to calves that did not receive the supplementation. Additionally, a positive impact of this bacterium on animals suffering from diarrhoea was observed. The researchers noticed that calf illness did not lead to a decrease in the average daily gain. However, there was a decrease in disruption to the faecal microbiota in supplemented calves compared to those that were not given supplements (Villot et al., 2019).

Blood morphological parameters serve as a crucial indicator of proper body homeostasis. The research found that the haematological parameters of the calves were within the reference ranges for healthy calves provided by Baumgartner (2005) and Knowles et al. (2000). The observed rise in red blood cell count on consecutive days of rearing aligns with the findings of Mohri et al. (2007) and Kupczyński et al. (2008), who reported a natural increase in erythrocyte count in the blood as calves get older. The decline in blood haemoglobin levels during the first weeks of life in new-born calves may be regarded as a physiological pattern

(Mohri et al., 2007). The leukocyte count on day 7 and day 60 in calves in the group treated with probiotics, using the highest values within the normal range, aligns with the findings of previous studies. Agazzi et al. (2014) discovered that when probiotics were given to calves, their blood profile followed the typical pattern seen in the early stages of development. These scientists discovered a slight decrease in lymphocytes, along with an increase in monocytes and neutrophils, in both the experimental and control groups during the whole trial. The group treated with probiotics showed a substantial increase in haemoglobin levels and eosinophil percentage on day 8, but the proportion of basophils fell towards the end of the research (Agazzi et al., 2014). Qadis et al. (2014) discovered that the presence of beneficial bacteria in the gastrointestinal tract may activate white blood cells in healthy calves. Additionally, the injection of probiotics further enhances this effect. Administering a probiotic made from bacteria may improve the function of cellular immunity in young calves after they have been weaned. According to Constable et al. (2001) calves exhibiting symptoms of diarrhoea have elevated levels of haematocrit, haemoglobin, and MCHC, while simultaneously displaying a drop in MCV. Conversely, a study conducted by Roodposhti and Dabiri (2012) found no correlation between the time of probiotic intake and the amounts of white blood cells, neutrophils, lymphocytes, and monocytes. In a similar vein, Omran et al. (2020) discovered that the utilisation of probiotics failed to have any notable impact on the parameters of white and red blood cells.

Colostrum plays a crucial role in transferring passive immunity to calves. Failure of passive transfer (FTP) leads to higher rates of calf illness and death (Vogels et al., 2013; Boccardo et al., 2016), reduced productivity, and an increased likelihood of being culled later in life (Faber et al., 2005). The quantities of IgG in calf serum progressively rise after the absorption of IgG from colostrum via the gastrointestinal tract. The highest amounts are typically recorded about 24-36 hours after feeding. On the other hand, the natural synthesis of immunoglobulin classes G and M starts between 1 and 2 weeks after birth, leading to a subsequent rise in IgG concentrations (Hassig et al., 2007). It is generally accepted that in calves who have received an adequate supply of colostrum, the concentration of immunoglobulin G in their blood on the second day of birth should be higher than 15 $g\text{-}l^{-1}$. However, values over 10 $g\text{-}l^{-1}$ are considered to be sufficient (Skrzypczak et al., 2011). An IgG level below 10 g/L shows a deficit in passive immune transfer (FTP). This scenario doubles the likelihood of sickness and increases four-fold the probability of calf death (McGee et al., 2005, 2006). The elevated levels of immunoglobulin concentrations reported in our experiment among calves in both studied groups suggest a successful transmission of colostrum immunity. The elevated levels of immunoglobulins observed in the tested groups that received the probiotic preparation align with the findings of Roodposhti and Dabiri (2012), who reported increased plasma IgG1 concentrations when using synbiotic and prebiotic preparations. However, it is important to note that the observed differences were not statistically significant. Yet, the studies conducted by Morrill et al. (1995) and Riddell et al. (2010) demonstrate that the addition of probiotics did not have a notable impact on the levels of immunoglobulins in calves. The peak IgG antibody concentrations seen on day 7 of life are caused by the transfer of antibodies from colostrum, leading to a subsequent drop in plasma IgG levels in calves until they are capable of producing their own antibodies (Riddell et al., 2010).

Lactic acid bacteria (LAB) are recognised as a means to preserve the equilibrium of gut microbial communities and inhibit the growth of potentially harmful bacteria. Lactic acid bacteria are believed to have antimicrobial effect against *Escherichia coli* and *Salmonella sp*., which are often used as microbiological markers of gut health. The decreased presence of *E. coli* bacteria in the faeces of calves that were administered the probiotic preparation aligns with the findings of previous studies (Elam et al, 2003; Roodposhti and Dabiri, 2012; Agazzi et al, 2014). Roodposhti and Dabiri (2012) demonstrated a decrease in the quantity of *E. coli* bacteria

in the group that was administered probiotics. The impact is further amplified when probiotics, prebiotics, and synbiotics are administered together. The reason for this is likely because probiotic microbes generate chemicals such as organic acids, hydrogen peroxide, and bacteriocins, which possess antimicrobial properties. As a result, they might potentially hinder the growth of some infections (Roodposhti and Dabiri, 2012). The positive impact is partly a result of the fight between probiotic bacteria and pathogenic bacteria for colonisation of the intestinal epithelial surface. A study conducted by Elam et al. (2003) showed that the presence of *Lactobacillus acidophilus* led to a decrease in *E. coli* bacteria in the faeces of oxen. Agazzi et al. (2014) demonstrated that the probiotic-treated group had a greater prevalence of *Lactobacillus* compared to *E. coli*, indicating a beneficial equilibrium in the gut microbiota. Abney's (2001) research, in contrast, found no statistically significant disparity in the quantity of E. coli present in the faeces of calves that were administered probiotics and prebiotics compared to the control group.

Prior to weaning, calves are vulnerable to many diseases that have the potential to impact their future performance. Coccidiosis is recognised as a significant contributing factor in diarrhoea in calves. The infection occurs when sporulated oocysts are ingested by contaminated feed, drink, or by licking infected surfaces. It often happens in environments with poor cleanliness, increased animal population, and stressful conditions like weaning (Verma et al., 2018). The incidence of *Eimeria sp.* infection is particularly high in calves, leading to the occurrence of clinical or subclinical coccidiosis. It is important to note that *Eimeria sp.* may be often detected in the faeces of healthy calves. Therefore, it is recommended to conduct clinical observations of diarrhoea and faecal testing for *Eimeria sp.* at the same time (Cornelissen et al., 1995). The agricultural business suffers a significant economic effect from both clinical and subclinical coccidiosis, mostly due to the expenses associated with treatment and the decreased productivity of affected animals.

Probiotics generally have a beneficial impact on various animal species by enhancing their performance. They play a role in shaping the composition of the intestinal microflora, resulting in a reduced occurrence of harmful microorganisms and improved utilisation of nutrients. The study's findings suggest that administering the probiotic preparation had a positive impact on the weight gain and final body mass of the calves. Additionally, it had a beneficial impact on the physical characteristics of blood, levels of immunoglobulins, and the composition of the gastrointestinal microflora and parasitic organisms.

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